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Short communication

Backbone ^{1}H, ^{15}N, and ^{13}C Resonance Assignments of the *Helicobacter pylori* Acyl Carrier Protein

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One of the small proteins from *Helicobacter pylori*, acyl carrier protein (ACP), was investigated by NMR. ACP is related to various cellular processes, especially with the biosynthesis of fatty acid. The basic NMR resonance assignment is a prerequisite for the validation of a heterologuous protein interaction with ACP in *H.pylori*. Here, the results of the backbone ^{1}H, ^{15}N, and ^{13}C resonance assignments of the *H. pylori* ACP are reported using double- and triple-resonance techniques. About 97% of all of the ^{1}HN, ^{15}N, ^{13}CO, $^{13}C\alpha$, and $^{13}C\beta$ resonances that cover 76 of the 78 non-proline residues are clarified through sequential- and specific- assignments. In addition, four helical regions were clearly identified on the basis of the resonance assignments.

Keywords: ACP, *H. pylori*, NMR

Introduction

Acyl carrier protein (ACP) is a small acidic protein that primarily plays a pivotal role in various biosynthesis. ACP is predominantly associated with the biosynthesis of fatty acid, but is also utilized in the synthesis of polyketide antibiotics, non-ribosomal peptides, and intermediates, such as the protein bound co-enzymes lipoic acid and biotin that are used in the synthesis of vitamins (Lynen *et al*., 1980; Shen *et al*., 1992; Sanyal *et al*., 1994; Kleinkauf *et al*., 1996). Because of its functional importance on bacterial viability, ACP is considered to be a potential target for the development of antibiotics. ACP exists in both an active (holo) and inactive (apo) form in which the activation of ACP is mediated by holo-acyl carrier protein synthase (ACPS), which transfers the 4PP-moiety of CoA to the 36th residue Ser of apoACP.

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The structures of ACPs are highly conserved in many bacterial strains. The three-dimensional structures of ACPs that originated from *E.coli* (Holak *et al*. 1988; Kim *et al*., 1989), *Streptomyces coelicolor* (Crump *et al*., 1997), *Bacillus subtilis* (Xu *et al*., 2001), and *Mycobacterium tuberculosis* (Wong *et al*., 2002) were previously determined by NMR studies. The complex model of holo-ACP and ACPS was also reported using X-ray crystallography (Parris *et al*., 2000). These reports showed that ACPs adopt a four α-helical bundle which are connected by loop regions of various lengths. While the biological functions of ACP and the structure of ACP itself have been investigated by many investigator groups, the mechanism of protein-protein interactions on the structural level is still unclear.

Here, we report the sequence-specific backbone resonance assignments of *Helicobacter pylori* ACP. This study on $ACP_{H.pylori}$ is the first step toward the investigation of heterologous protein interactions since $ACP_{H.pylori}$ is related to various cellular processes and interacts with many different enzymes, including functionally unknown proteins in *Helicobacter pylori*.

Materials and Methods

H. pylori ACP (HP0559, 78 a.a) was cloned from a genomic DNA from *H. pylori* (ATCC 700392) using PCR and placed into a pET 21-a vector (Novagen). Recombinant, uniformly ^{15}N (>95%), ^{13}C (>95%) labeled $ACP_{H.pylori}$, was expressed in the *E. coli* strain BL21 (DE3), (Park *et al*., 2002) and the soluble protein was purified by His-tag affinity column followed by DEAE anion-exchange chromatography. The purified protein was treated with 50 mM DTT at 37°C overnight to remove acyl chains. The DNA of the expression clone was sequenced and the purity of the proteins was determined by SDS-PAGE and mass spectrometry.

A 500 μl NMR sample was prepared containing 4 mM $ACP_{H.pylori}$, 50 mM sodium phosphate buffer, 1 mM EDTA, 1 mM DTT, 0.02% sodium azide, 500 mM NaCl, 95% H_2O/5%D_2O at pH 6.0. All spectra were recorded on Bruker DRX 500 and 600 NMR spectrometers at 303 K (Ammar *et al*., 2002). Assignments were

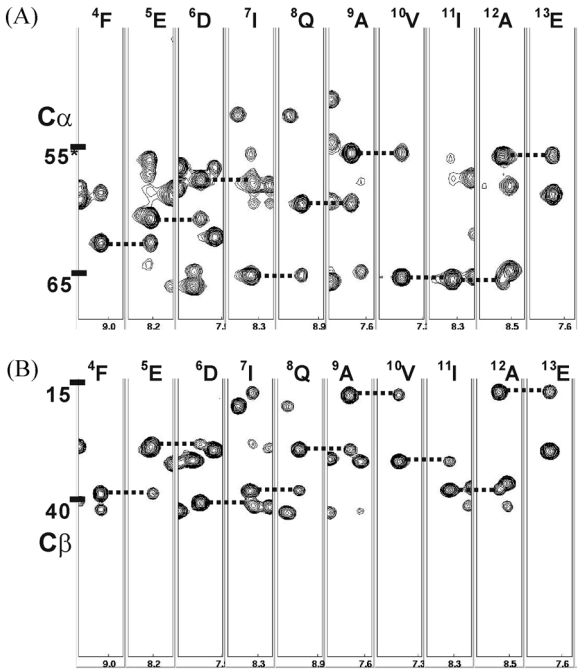

Fig. 1. Strip plots of $ACP_{H.pylori.}$ (residues 4-13) (A) Sequential connectivity of Cα carbons is depicted in HNCA spectrum. The dotted lines represent the intermolecular connectivity. (B) Sequential connectivity of Cβ carbons in HNCACB spectrum. (*unit, ppm)

derived from standard and TROSY versions of HN(CO)CA, HN(CO)CACB, HNCACB, HNCA, HNCO, ^{15}N edited NOESY, and ^{15}N edited TOCSY experiments. The ^{1}H, ^{15}N, and ^{13}C chemical shifts were referenced relative to the frequency of the methyl proton resonance of DSS. All data were processed using NMRPipe/ NMRDraw. Typically, a sine-bell function was applied to the time domain data before zero filling.

Results and Discussion

The native $ACP_{H.pylori}$ contains 78 amino acids with a molecular weight of 8 kDa. The assignments comprise 97% of all ^{1}HN, ^{15}N, ^{13}CO, $^{13}C\alpha$, and $^{13}C\beta$ resonances covering 76 of the 78 non-proline residues. Two residues are not observed in a 2D ^{1}H-^{15}N HSQC spectrum; therefore, they were not assigned (residues 1 and 2). Missing peaks are presumed to be from residues in the intermediate conformational exchange and consequently broadened beyond detection. The sequential connectivity of Cα and Cβ carbons in the first helical region is shown in Fig. 1. On the basis of resonance assignments, four helical regions were clearly identified using the CSI Program (Wishart *et al*., 1994, data not shown). These correspond to residues 3L-Q14 (αI), S36-G51 (αII), D56-E60 (αIII), and V65-K76 (αVI), which is consistent with previous reports on other bacterial ACPs. Therefore, the tertiary structure of $ACP_{H.pylori}$ appears to be quite similar to those of other bacterial ACPs. All of the ^{1}HN, ^{15}N, ^{13}CO, $^{13}C\alpha$, and $^{13}C\beta$ resonances are represented in Table 1. Figure 2 shows the ^{1}H-^{15}N HSQC

Table 1. Chemical shifts of ^{1}HN, ^{15}N, ^{13}CO, $^{13}C\alpha$ and $^{13}C\beta$ of $ACP_{H.pylori}$ All the Chemical shifts were referenced relative to the frequency of the methyl proton resonance of DSS

Residue	HN	N	CO	CA	CB
1MET	ND*	ND	ND	ND	ND
2ALA	ND	ND	174	51.9	18.45
3LEU	8.49**	122.42	178.31	58.59	41.67
4PHE	9.02	116.95	176.12	62.95	38.48
5GLU	8.2	117.76	179.41	60.76	28.27
6ASP	7.98	121.63	ND	57.45	40.34
7ILE	8.32	122.68	177.46	65.78	37.87
8GLN	8.97	121.28	177.08	59.54	29.38
9ALA	7.65	118.43	180.62	55.15	18.26
10VAL	7.38	118.83	178.06	65.94	31.84
11ILE	8.31	119.26	176.89	66.16	37.71
12ALA	8.53	120.49	179.71	55.38	17.55
13GLU	7.64	115.55	179.24	58.77	29.71
14GLN	8.51	117.57	177.55	58.18	28.86
15LEU	8.24	112.75	175.99	54.12	41.07
16ASN	7.74	117.64	174.42	54.22	36.76
17VAL	7.97	110.45	174.42	58.54	34.71
18ASP	8.14	121.62	177.92	54.4	42.16
19ALA	8.75	127.91	179.43	56.05	17.7
20VAL	8.16	112.56	177.55	64.22	31.24
21GLN	7.8	116.73	175.85	56.45	29.83
22VAL	7.92	121.8	173.17	62.41	29.51
23THR	7.02	115.9	ND	58.32	68.02
24PRO			177.37	65.75	31.8
25GLU	8.2	111.71	176.44	56.62	29.1
26ALA	7.71	125.96	176.48	52.97	18.22
27GLU	10.35	122.68	178.93	55.2	30.52
28PHE	7.32	121.7	175.45	62.54	38.37
29VAL	8.47	116.94	179.34	64.9	31.19
30LYS	8.21	117.31	177.32	60.3	33.27
31ASP	7.47	110.95	177.53	56.26	42.06
32LEU	7.38	115.18	176.97	54.51	41.36
33GLY	7.36	105.91	174.3	46.6	ND
34ALA	8.38	123.27	177.19	51.78	20.52
35ASP	9.01	120.71	177.45	51.95	42.38
36SER	8.4	114.07	176.77	62.5	63.14
37LEU	8.03	123.25	178.97	57.6	40.91
38ASP	8.08	119.8	179.64	57.17	41.11
39VAL	7.74	119.49	178.39	67.41	31.08
40VAL	7.46	120.1	178.92	67.11	31.46
41GLU	7.98	118.4	179.27	59.46	28.93
42LEU	8.28	122.85	177.79	57.96	41.04
43ILE	8.5	120.47	178.04	65.4	36.3
44MET	7.75	117.66	178.94	59.35	32.85
45ALA	7.97	122.66	181.11	55.01	17.68
46LEU	8.63	119.48	179.53	58.01	42.03
47GLU	8.72	120.54	179.84	60.02	29.91
48GLU	7.82	117.92	178.77	58.96	29.79
49LYS	8.1	117.5	178.05	58.63	32.25
50PHE	8.15	111.49	175.92	58.77	39.39

Table 1. Continued

Residue	HN	N	CO	CA	CB
51GLY	7.79	109.1	174.28	47.41	ND
52VAL	7.46	113.76	173.8	59.17	34.79
53GLU	8.38	123.55	175.84	55.53	31.07
54ILE	9.53	127.93	ND	58.64	37.36
55PRO			177.46	62.55	32.67
56ASP	8.84	124.32	177.89	58.13	40.09
57GLU	9.12	116.65	178.16	59.22	28.68
58GLN	7.27	116.33	178.08	56.77	28
59ALA	8.32	123.37	179.6	55.24	17.84
60GLU	7.65	113.39	176.79	57.85	29.64
61LYS	7.34	115.81	176.3	55.51	32.79
62ILE	7.29	121.55	173.95	62.44	37.43
63ILE	9.27	128.3	176.76	61.53	39.94
64ASN	8.68	119.04	174.59	50.49	42.42
65VAL	7.74	118.44	178.15	66.31	31.37
66GLY	9.25	108.76	176.05	47.03	ND
67ASP	8.51	122.08	179.11	57.56	42.08
68VAL	7.61	119.03	176.28	65.44	31.93
69VAL	8.01	121.66	177.1	66.72	31.63
70LYS	8.12	117.37	177.82	59.01	32.1
71TYR	7.52	116.64	178.89	62.2	38.42
72ILE	8.2	119.23	177.38	65.87	37.04
73GLU	8.76	120.13	181.11	60.17	29.66
74ASP	8.53	117.5	178.34	56.6	40.31
75ASN	7.62	116.99	175.81	55.46	39.95
76LYS	8.14	120.91	177.72	58.26	32.24
77LEU	8.08	121.09	178.06	56.4	41.89
78ALA	7.89	122.84	178.46	53.36	18.61

*ND; not detected
** unit; ppm

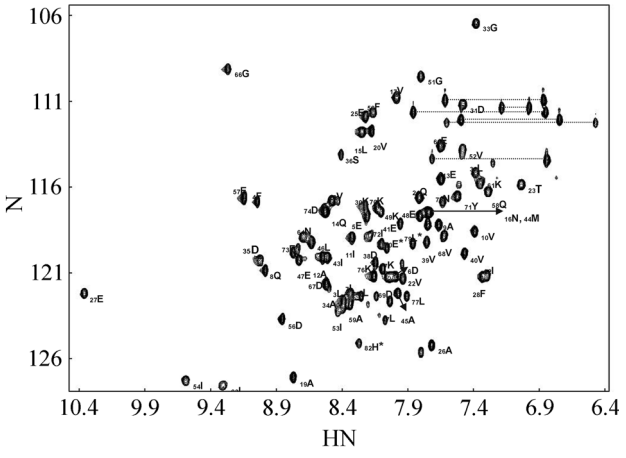

Fig. 2. HSQC spectrum of $ACP_{H.pylori.}$ The cross peaks in the spectrum are labeled with the assigned amino acid residue. The dotted lines represent the sidechains of Gln and Asn. The additional residues originated from the vector sequence are indicated by the asterisk.

spectrum that is labeled with the assigned amino acid residue. These data will be useful in studying the heterologous protein interactions in *H. pylori*.

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